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Importins/Karyopherins Meet Nucleoporins

Meeting Review

E. C. Hurt
Universität Heidelberg
Institut für Biochemie I
Im Neuenheimer Feld 328
D-69120 Heidelberg
Federal Republic of Germany

The Fourth International Symposium on Nuclear–Cytoplasmic Transport held October 1–4, 1995, at the German Cancer Research Center in Heidelberg showed that this research field is currently providing novel insights into the mechanism of nucleocytoplasmic transport and the organization of the nuclear pore complex (NPC). Besides research dealing with nuclear pore structure and function, other aspects concerning the functional organization of the cell nucleus into subcompartments and certain aspects of ribosome biogenesis were discussed at this meeting, which had the following topics: molecular anatomy of the nuclear periphery, nucleocytoplasmic protein transport and its impact on transcription and differentiation, nucleocytoplasmic transport of ribonucleoproteins, functional topology of the cell nucleus, nuclear compartmentation, and ribosome biosynthesis. In this meeting review, I will focus mainly on presentations dealing with the NPC and nucleocytoplasmic transport and only summarize the essence of the other topics.

In his historical overview on nucleocytoplasmic transport, R. Laskey (Wellcome/Cancer Research Center Institute, Cambridge, United Kingdom) emphasized that several phases of research were crucial for the development of this field. Early on, microinjection studies in Xenopus oocytes revealed that nuclear proteins have the capability to specifically accumulate inside the nucleus (reviewed by Dingwall and Laskey, 1986). The second phase began when nucleoplasmin and SV40 large T antigen were analyzed for their nuclear localization signals (NLSs), leading to the concept that nuclear location is a local and not a global property of the karyophilic protein. At about the same time, Carl Feldherr saw in the electron microscope that microinjected gold particles coated with nucleoplasmin were lining up on short NPC attached filaments upon transit through the pore channel. A third phase began with the development of in vitro assays, using initially the Xenopus nuclei reconstitution system (Newmeyer et al., 1986a, 1986b) and later the digitonin-permeabilized mammalian cells (Adam et al., 1990). These assays revealed that nuclear protein transport is a two-step mechanism in which the substrate first binds to the nuclear envelope (most likely to the pore fibrils) and then is translocated through the pore channel by an energy-dependent mechanism. Consequently, the permeabilized system in combination with fractionated cytosol was used to purify soluble factors required for nuclear import (Moore and Blobel, 1992; Melchior et al., 1993; Görlich et al., 1994). We are now beginning to understand what these distinct steps mean in terms of the molecular mechanism of translocation (see also below). In parallel to this functional analysis, the biochemical dissection of the NPC was also pursued with the goal of identifying the numerous nuclear pore proteins and studying their role in nucleocytoplasmic transport. Recently, the purification of milligram quantities of highly enriched yeast NPCs and a genetic dissection of the NPC gave a burst of knowledge on the nature of nuclear pore proteins and factors involved in nucleocytoplasmic transport (reviewed by Rout and Wente, 1994; Doye and Hurt, 1995).

The Architecture and Biogenesis of the NPC

Electron microscopy (EM) analysis of the NPC over the past 25 years has provided a detailed structural picture of this 125 MDa macromolecular assembly. The NPC consists of four basic elements: the spoke–ring complex, the central plug ("transporter"), cytoplasmic fibrils, and the inner nuclear basket. C. Akey (Boston University, Boston, Massachusetts) reported on a three-dimensional (3D) EM analysis of frozen/hydrated yeast NPCs in ice. Accordingly, yeast NPCs are physically smaller (about half the size in height and significantly smaller in diameter) than higher eukaryotic NPCs, suggesting that some structures are absent. In particular, yeast NPCs seem to lack a luminal spoke domain, which in vertebrate NPCs has been attributed to the luminal domain of gp210 (Jarnik and Aebi, 1991), raising the question of whether yeast lack a homolog to murine gp210. In contrast, the inner spoke ring and the central transporter of yeast NPCs highly resemble the corresponding structures in metazoans. Thus, different substructures of the NPC may have evolved independently. The ability to perform 3D reconstructions of NPCs will make it possible to map the relative positions of nuclear pore proteins within the overall NPC framework. Furthermore, since yeast mutants lacking a single NPC protein are often viable, 3D maps of mutant and wild-type NPCs can be compared. This should give a better understanding of where nucleoporins are localized with respect to the known NPC substructures and how they contribute to the overall structure.

M. Rout (Rockefeller Institute, New York) analyzed the yeast NPC from a biochemical point of view by characterizing the NPC proteins derived from his highly purified yeast NPC preparation. This biochemical work has progressed so quickly that a total of 19 nucleoporins have already been identified, which may correspond to as many as 50% of all yeast NPC proteins (Rout and Blobel, 1993). Of these components, Nup188p, Nup170p, Pom152p, Nup156p, and Nic96p are the most abundant ones, contributing to about 25% of the total NPC mass. Nup170p and Nup157p, which are highly homologous and form a functional pair, are genetically linked to Nup188p and Pom152p and have a mammalian counterpart in Nup155p, which can complement synthetic lethal mutants of Nup170p/Pom152p (Aitchison et al., 1995b). This result gives rise to the hope that vertebrate NPC proteins can be functionally analyzed in an in vivo system amenable to sophisticated genetic manipulations.

Having so many nucleoporins in hand, it is important

to put them in a functional and topological context. S. Siniossoglou (Universität Heidelberg, Federal Republic of Germany) addressed this question by studying how yeast nucleoporins physically interact with each other. He discovered a novel subcomplex of the yeast NPC that contains, besides several nucleoporins (Nup120p, Nup85p, and Nup84p), an unexpected protein, Sec13p, for which a role in endoplasmic reticulum (ER) to Golgi transport has been established (Pryer et al., 1993), plus a homolog of Sec13p called Seh1p. Mutants lacking Nup84p, Nup85p, or Nup120p exhibit abnormalities in nuclear membrane and NPC organization and are impaired in poly(A)$^+$ RNA export. This was also reported by M. Rout, who identified Nup120p as a constituent of the yeast NPC preparation (Aitchison et al., 1995a). The fact that Sec13p, a subunit of the COPII coatomer complex, is also found in this novel nucleoporin complex raises the intriguing possibility that factors participating in membrane biogenesis and vesicular transport may also function in nuclear envelope and NPC formation.

To find out about these putative common steps, K. Ullman (University of California, San Diego, La Jolla, California) studied whether the assembly of NPCs in Xenopus synthetic nuclei occurs concomitantly with nuclear envelope assembly. She showed that N-ethylmaleimide-treated membranes could still bind to chromatin in a reconstitution assay, but attached vesicles did not fuse, and accordingly, nucleoporins were not associated. Interestingly, GTPγS inhibited both nuclear envelope fusion and nuclear pore assembly. It is yet not clear why there is GTP dependence for NPC formation, but it could be that specific vesicles are also required for this step. Interesting in this context is that D. Lourim (Universität Würzburg, Federal Republic of Germany), who analyzed the composition of mitotic vesicles, found at least four different vesicle populations that are involved in the formation of the nuclear envelope. Whereas each of the three Xenopus B-type lamins are associated with separate vesicle populations, the fourth type of vesicle specifically contains a Xenopus integral pore membrane protein related to mammalian gp210, but lacks peripheral nucleoporins such as p62. These data raise the possibility that specific vesicles containing a pore membrane protein are involved in nuclear pore and nuclear membrane biogenesis.

An alternative system for studying NPC formation independent of nuclear envelope and nuclear lamina biogenesis was reported by V. Cordes (Deutsches Krebsforschungszentrum, Heidelberg). He analyzed nuclear pore proteins in the cytoplasmically located annulate lamellae (AL) of Xenopus oocytes that form membrane stacks with multiple NPCs. Several nuclear pore marker proteins (e.g., p62, gp210, and RanBP2) are present in AL (see also Meier et al., 1995), but nucleoporins that are exclusively located on intranuclear pore filaments (e.g., Nup153) are absent. AL also abundantly occur in different somatic cells in which they assemble/disassemble during mitosis nearly concomitantly with the nuclear envelope and the rough ER. Total cell overviews revealed that AL frequently are located in continuity with the rough ER; whether AL, however, are derived from rough ER or are merely interconnected is not clear. Since AL can bind NLS substrates (Feldherr et al., 1984), it remains open as to whether they bind the soluble factors involved in nuclear transport (e.g., Ran/TC4), thereby fulfilling a storage or regulatory function, or whether they play a role in NPC/nuclear envelope biogenesis.

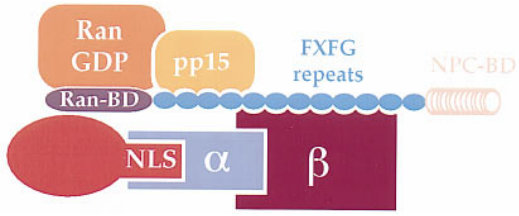

Figure 1. A Model of Interaction among Components Involved in Nucleocytoplasmic Transport

It is not clear whether the drawn interactions occur simultaneously. NLS, karyophilic protein with a nuclear localization sequence; α, karyopherin-α/importin-α; β, karyopherin-β/importin-β; RanGDP, Ran/TC4 in its GDP-bound form; pp15, nuclear import factor pp15; nucleoporin with Ran-binding domain (Ran-BD), FXFG repeat sequences (FXFG), and an NPC-binding domain (NPC-BD). For further explanations, see text.

For the further study of nuclear pore formation, it will be useful to be able to visualize single NPCs by fluorescence microscopy. U. Kubischek (Universität Münster, Federal Republic of Germany) applied the single particle tracking methodology using confocal microscopy to visualize and localize individual NPCs in permeabilized cells with an accuracy of a few nanometers. This is an important step forward toward a quantitative analysis of NPC formation, but this method can also be used to study the transport capability of single nuclear pores.

The Mechanism of Nuclear Protein Import

As already pointed out, the roles of the NLS receptor complex (also called importin-α/β or karyopherin-α/β, as well as Srp1p in yeast) and the GTPase cycle of Ran in the two distinct steps of nucleocytoplasmic transport are becoming more and more transparent (see Figure 1) owing to the availability of in vitro nuclear transport assays (D. Görlich, Wellcome/Cancer Research Center Institute; M. Moore, Baylor College of Medicine, Houston, Texas; U. Nehrbass, Rockefeller Institute; M. Nomura, University of California, Irvine; K. Weis, European Molecular Biology Laboratory, Heidelberg). As M. Moore found, two fractions are sufficient to reconstitute nuclear protein import in vitro (Moore and Blobel, 1992). Fraction A consists of a nuclear envelope docking activity for which the importin–karyopherin-α/β complex is responsible, whereas fraction B is composed of the GTP-binding protein Ran/TC4 and a stimulating factor called pp15, which is associated with Ran. If an import substrate plus fraction A is added to the in vitro assay, nuclear envelope binding is observed. Addition of GTP, Ran, and pp15 triggers a chase of the karyophile into the nucleus. The nonhydrolyzable GTP analog GMP–PNP bound to Ran causes the karyopherin–karyophile complex to come off the nuclear envelope and to remain in the cytoplasm.

D. Görlich found in the semiopen cell system that recombinant importin-α, importin-β, and Ran alone are sufficient to restore nuclear import. The binding of the

import substrate to the NPC only occurs when both importin-α and importin-β form a complex; the subsequent translocation into the nucleus requires addition of Ran and energy. The karyophile and importin-α finally accumulate in the nucleoplasm, whereas importin-β stays behind at the NPC (Görlich et al., 1995). Importin-α binds via its N-terminus to the β subunit (similar data were also provided by K. Weis; see below). This N-terminus functions as an archetypal nuclear targeting sequence that is specifically and only recognized by importin-β. The hydrophobic middle domain of importin-α consisting of eight *armadillo* (*arm*) repeats is implicated in NLS binding. As the domain of importin-α that binds importin-β is characterized by clusters of basic amino acids like an NLS, one might assume that the two motifs are recognized in a similar way. Thus, the NLS-like N-terminus of importin-α might be bound by the *arm*-like motifs in importin-β.

K. Weis found a human *SRP1* homolog (importin–karyopherin-α) in a two-hybrid screen using p80 coilin (a marker protein of the coiled bodies; see also below) as bait, most likely as a result of a physical interaction between the coilin NLS and the NLS receptor (Weis et al., 1995). In fact, if mixed with recombinant hSRP1α, a NLS–bovine serum albumin conjugate specifically bound to the NLS with a binding constant of 3×10^{-9} M for NLS–hSRP1α receptor complex. Since so many isoforms of hSRP1α have been already identified (with homologies in the 50% range), the various NLS receptors may not completely functionally overlap, but could be involved in recognizing different types of NLSs. Interestingly, certain hSRP1α isoforms can be expressed tissue specifically, suggesting a specific role in differentiation and organogenesis.

The yeast NLS receptor α subunit called Srp1p (Yano et al., 1992) is physically associated not only with karyopherin-β, but also with nucleoporins such as Nup1p and Nup2p (Belanger et al., 1994). Many thermosensitive alleles of *SRP1* have been isolated, as pointed out by M. Nomura, which cause different phenotypes, including reduced nuclear protein import and impaired binding to NLS peptides, nucleolar fragmentation, decrease in transcription, chromosomal instability, defects in nuclear division, and microtubule morphology. This raises the question whether Srp1p has, in addition to its NLS receptor function, other roles. It is still not clear how the NLS receptor is released from the karyophilic substrate after transport into the nucleus, but phosphorylation/dephosphorylation could be the trigger. Interestingly, an Srp1p kinase was partially purified from yeast, whose activity is stimulated by addition of NLS or karyophilic proteins. Thus, the Srp1p kinase is a candidate protein that could control binding or release of karyophilic proteins upon phosphorylation of the NLS receptor.

One of the central problems is how the Ran cycle works to achieve the transport of the karyophile over a 100 nm distance through the pore channel. This was addressed by several speakers during the meeting (A. Dickmanns, Institut Biochemie, Universität München, Federal Republic of Germany; F. Melchior, University of California, San Diego; M. Moore; U. Nehrbass; H. Ponstingl, Deutsches Krebsforschungszentrum). As reported by H. Ponstingl, many functions have been attributed to Ran/TC4 as well as to its Ran-specific guanine dinucleotide exchange factor RCC1, including regulation of chromosome segregation, DNA replication, cell cycle checkpoint control, RNA synthesis and processing, nuclear import and export, and nuclear envelope formation. The question therefore remains as to whether Ran, RCC1, or both are directly involved in all these processes. Since RCC1 is only found in the nucleus and RanGAP1 in the cytoplasm, the two main factors affecting the Ran cycle are located in two different compartments. This topological problem still represents a conceptual enigma to describe precisely how Ran works in nucleocytoplasmic transport. Accordingly, many questions concerning the Ran function remain open, such as how RanGTP is exported into the cytoplasm, whether cytoplasmic RanGTP is protected from GTP hydrolysis, whether Ran is also required for RNA export, why RCC1 mutants reach the G2/M boundary and do not stop earlier in the cell cycle, and whether RanGTP has additional nuclear functions. F. Melchior used mutant forms of Ran to address some of these questions. The permanently activated Ran Q69L mutant, which cannot hydrolyze GTP, still accumulates at the nuclear envelope, but cannot trigger nuclear protein import. This may suggest that the nuclear envelope is the place where Ran hydrolyzes GTP. Also, the nonhydrolyzable RanGDP–PNP can dock at the NPC (in contrast with RanGDP) at sites which are about 50–60 nm away from the center of the NPC; these may be the sites at which import substrates also bind and may structurally correspond to the outer pore fibrils. Interestingly, a Ran-binding protein called RanBP2 (Yokoyama et al., 1995) or Nup358p is located at these pore fibrils (Wu et al., 1995; Wilken et al., 1995). Thus, Nup358p is a good candidate for concentrating Ran at the NPC.

U. Nehrbass found another NPC protein, Nup36p, that also contains, besides repeat sequences, a Ran-binding domain. He reported that in an in vitro assay the karyopherin-α/β complex interacts with FXFG repeats of Nup36p in a cooperative fashion. Only the karyopherin-α/β complex, but not the single subunits, bind to FXFG repeats. This binding is specific for the FXFG repeats (see also Rexach and Blobel, 1995). RanGTP (but not RanGDP) was able to dissociate the karyopherin-α/β complex from the FXFG repeats, mimicking an undocking reaction that may also occur in vivo. Another factor that seems to be involved in targeting Ran to the trimeric complex is the yeast pp15 homolog. As discussed above, vertebrate pp15 is a Ran-associated nuclear import factor (Moore and Blobel, 1994; Paschal and Gerace, 1995). U. Nehrbass showed that yeast pp15 is essential for cell growth, is localized to the nuclear periphery, and can bind to several repeat-containing nucleoporins. He could demonstrate that in the in vitro reaction recombinant pp15 allowed stable binding of RanGDP to the trimeric karyopherin-α/β–Nup36p complex, and addition of GTP to the prebound karyopherin-α–karyopherin-β–RanGDP–pp15 complex caused the release of the α subunit.

One of the still unsolved questions concerning the mechanism of nuclear protein import is how force is generated to move the karyopherin–karyophile complex over a distance of 100 nm from the *cis* to the *trans*

side of the NPC. For this movement, interaction of the karyopherin–karyophile complex with NPC components along the transport channel must occur. This could happen by many reiterated steps of binding and release along a track of repeat-containing nucleoporins. If along these tracks the affinity of the karyopherin–karyophile complex to nucleoporin repeats can increase, the transport substrate could be driven into the nucleus without a direct force-generating device. As discussed by U. Nehrbass, an ordered arrangement of nucleoporins with increasing affinities to the karyopherin–karyophile complex along the transport axis could be achieved, e.g., by varying the number of repeat sequences within a given nucleoporin along the transport route.

The Mechanism of Nuclear RNA and Protein Export

Several classes of RNA are transported from the nucleus to the cytoplasm, including messenger RNA (mRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), small nuclear RNA (snRNA), or, in the case of viral assembly, viral mRNA (e.g., HIV mRNA). RNA transport starts with transcription and then involves packaging (e.g., association with RNA-binding proteins), transport to the nuclear periphery, and export through the NPC. Our view on how RNA is transported out of the nucleus was changed during this year when several groups identified nuclear export signals (NES) in nuclear factors implicated to be involved in RNA export (reviewed by Gerace, 1995). Cellular mRNAs are only exported when splicing is completed inside the nucleus. U. Fischer (Universität Marburg, Federal Rebublic of Germany) reported on the role of the HIV Rev protein in the nuclear export of unspliced and partially spliced viral mRNA (Fischer et al., 1995). A short sequence, LPPLERLTL, within the activator domain has now been shown to be an NES, demonstrating that Rev is a nuclear export factor that interacts with a cellular export factor that does not participate in the export of cellular mRNA, but seems to mediate the export of 5S rRNA and snRNA. The sequence within the activator domain of Rev, which acts as an NES, also occurs in other proteins (Wen et al., 1995), including Xenopus TFIIIA. Interestingly, this sequence within TFIIIA can functionally replace the corresponding NES within Rev. Since TFIIIA is involved in 5S rRNA export in Xenopus, it could act like the Rev protein. T. Pieler (Universität Göttingen, Federal Republic of Germany) has further elucidated the role of Xenopus TFIIIA, as well as the ribosomal protein L5 in the nucleocytoplasmic transport of 5S rRNA. The analysis of mutant forms of 5S rRNA revealed that TFIIIA and L5 act as export and shuttling factors, respectively, for 5S rRNA. Since TFIIIA is a DNA/RNA-binding protein, it needs to be retained in the cytoplasm for its function. This retention is mediated by the RNA-binding domain of TFIIIA located between zinc fingers 5 and 6 (involved in RNA binding). Accordingly, the 5S rRNA-mediated retention of TFIIIA in the cytoplasm adds to several other mechanisms of cytoplasmic retention of nuclear proteins (see also below); e.g., NF-κB, which interacts with IF-κB (Henkel et al., 1992), thereby masking the NLS or SWI5 in which cell cycle–regulated phosphorylation inactivates the NLS (Moll et al., 1991).

One of the cellular factors with which NESs interact was recently identified both in yeast and higher eukaryotes and is a protein with homology to repeat-containing nucleoporins (Bogerd et al., 1995; Stutz et al., 1995). Another cellular factor possibly involved in RNA export was purified by E. Izaurralde (European Molecular Biology Laboratory). It is the cap-binding protein (CBP) complex, which binds to the capped 5′ end of snRNA and mediates snRNA nuclear export (Izaurralde et al., 1995). This complex which is composed of CBP80 and CBP20 is located in the nucleus. Inhibition studies using anti-CBP20 antibodies showed that the CBP complex is directly involved in snRNA export. It may be that the CBP complex also contains an NES that allows interaction with NPC components through a similar mechanism to the Rev protein. Interestingly, CBP20 was found attached to the Balbiani ring pre-mRNA particles as seen by immuno-EM at a stage when the particle is in the process of being translocated through the NPC channel. The CBP complex thus appears to accompany the RNA on its way from the nucleoplasm to the NPC. Components that are required for RNA export were also identified through genetic screens in yeast. A. Tartakoff (Case Western Reserve University, Cleveland, Ohio), who found yeast mutants defective in poly(A)$^+$ RNA export (called *mtr* mutants; Kadowaki et al., 1992), reported on a further novel *mtr* mutant (*mtr4*) derived from this screen. The *mtr4* mutant phenotype is similar to three other *mtr* mutants that accumulate poly(A)$^+$ RNA not in the nucleoplasm, but inside the nucleolus. This could imply that RNA is rerouted through the nucleolus during export, in the case that the normal route through the nucleoplasm is inhibited. Mtr4p is a nuclear DEAD box–containing putative RNA helicase. It thus may have an RNA-unfolding activity to keep the RNA in a transport-competent conformation.

A major limitation in studying the mechanism of nuclear RNA export is the lack of a reliable in vitro system that can be experimentally manipulated. K. Ullmann reported on the use of synthetic nuclei to approach transcription and nuclear export of RNA in vitro (Ullman and Forbes, 1995). Previously, it was not clear whether reconstituted nuclei derived from Xenopus egg extracts and DNA are active in transcription and RNA export. To test for this, cloned DNA encoding 5S rRNA or tRNA was microinjected, and transcripts were radiolabeled. This in vitro transcription was not inhibited by polymerase III inhibitors, which cannot penetrate the nuclear membrane, showing that RNA was transcribed within an intact nucleus. Future plans with this system are to test whether the newly synthesized RNA can be exported and factors be found that are involved in RNA export reactions.

Regulated Nuclear Transport and Signaling through the NPC

Regulated nuclear transport is an efficient way to achieve controlled and rapid nuclear entry of particular proteins that are involved in signal transduction pathways from the plasma membrane to the nucleus (Nigg et al., 1991). Several authors addressed this aspect at the meeting, including regulated nuclear entry of Dorsal

(DL) (A. Bergmann, Max-Planck-Institut für Entwicklungsbiologie, Tübingen, Federal Republic of Germany), myoD (R. Rupp, Max-Planck-Institut für Entwicklungsbiologie), the progesterone receptor (E. Milgrom, Institut National de la Santé et de la Recherche Médicale, Le Kremlin-Bictre, France), and MAP kinase (A. Nordheim, Medizinische Hochschule, Hanover, Federal Republic of Germany). A nuclear localization gradient of DL determines the pattern of the dorsoventral axis within the embryo. In a wild-type embryo, DL is cytoplasmatic at the dorsal side and nuclear at the ventral side. In ventralized mutants and dorsalized mutants, DL is uniformly nuclear and cytoplasmatic, respectively. DL is found at the end of a complex signal transduction cascade involving many gene products that starts in the vitelline membrane and ends in the cytoplasm with Tube, Pelle, and Cactus (CACT). CACT is an IF-κB homolog with six ankyrin repeats and a PEST sequence for proteolytic degradation. Binding of CACT to DL may mask the DL NLS, thereby anchoring DL in the cytoplasm. CACT degradation is the trigger that releases DL, allowing its import into the nucleus. Similarly, the asymmetric nuclear accumulation of MyoD along an animal/vegetal axis in skeletal muscle cells (which initiates and executes muscle differentiation in early stages of embryo development) is also the result of regulated nuclear protein uptake of MyoD. This is controlled by a domain within MyoD that causes cytoplasmatic retention and thus overrides the function of the MyoD NLS. The proteins that cause cytoplasmatic retention are not known. Steroid hormone receptors also shuttle between the nucleus and the cytoplasm in a regulated manner. The progesterone receptor contains both a constitutive and a hormone-regulated NLS. If the constitutive NLS is deleted, the receptor follows a strictly hormone-dependent nuclear import, and it shuttles between the cytoplasm and nucleoplasm.

Nucleolar Targeting

Little is known about the mechanism that determines the location of nuclear proteins to specific intranuclear compartments. The nucleolus is the place where coupled rRNA transcription and processing and ribosome biogenesis take place (A. Hadjiolov, Centre National de la Recherche Scientifique, Toulouse, France). M. Schmidt-Zachmann (Deutsches Krebsforschungszentrum) analyzed the signals required for nucleolar targeting of Xenopus nucleolar NO38. Nucleolar targeting domains do not share a consensus sequence, and multiple regions are required for efficient nucleolar accumulation. Thus, nucleolar targeting is a two-step process involving an NLS-mediated nuclear import and nucleolar retention by virtue of interaction with nucleolar components. In the case of the human ribosomal protein S6, the structural integrity of the S6 protein is likewise required for nucleolar accumulation, supporting a model in which nucleolar retention contributes to nucleolar targeting of ribosomal proteins (J. Kruppa, Universität Hamburg, Federal Republic of Germany). However, for viral nucleolar proteins it was found that short sequences of 15 residues in length (considered to be an "extended" NLSs) can, in fact, constitute a bona fide nucleolar targeting sequence (reviewed by Schmidt-Zachmann and Nigg, 1993). Interestingly, several nucleolar proteins that are acidic and nonribosomal nucleolar proteins, such as NSR1, nucleolin, NO38, Nopp140, and NPI46, have been identified in the past as NLS-binding proteins; however, these proteins may not be directly involved in nuclear protein import, but have diverse roles in ribosome biogenesis (T. Mélèse, Columbia University, New York; Xue and Mélèse, 1994).

Intranuclear Structures and Transport

Confocal laser scanning microscopy-based fluorescent in situ hybridization has greatly improved the ability to detect genes and transcripts inside the nucleus. This method is a powerful tool to study not only the nuclear topography of genes, splicing snRNPs, mRNA transcripts, and chromosome domains by 3D reconstruction, but also where and how transport of cargo molecules occur inside the nucleus. P. Lichter (Deutsches Krebsforschungszentrum) reported on the territorial organization of the chromosomes inside the nucleus. When probes specific for the dystrophin, β-myosin, and β-globin genes were used, although independent of the activity of the gene, they appear to be peripheral to the chromosome territories. The fact that nuclear RNA transcripts appear on tracklike structures could indicate a transport of RNA toward the NPC. One aspect of future research will concentrate on whether these tracks are directly connected to the NPCs. Between the chromosome domains, a 3D network-like compartment, the interchromosome domain compartment, was analyzed by C. Cremer (Universität Heidelberg). In this space, many reactions, such as DNA replication and repair, RNA transcription, modification, splicing, and transport, may take place. Improvement of 3D microscopy is important for a further investigation of the chromosome territories and the interchromosome domain compartment. How these and other intranuclear structures form is not yet understood. P. Bell (Universität Würzburg) addressed this question by studying the assembly of prenucleolar bodies (PNBs) in an in vitro system using Xenopus egg extracts. The in vitro assembled PNBs contain nucleolar proteins such as fibrillarin, nucleolin, NO38, and the 180 kDa protein (XNopp180), as well as U1–U8 snRNAs. The nucleolar 180 kDa protein, nucleolin, fibrillarin, NO38, as well as U3 snRNA, proved to be not essential for the in vitro assembly of PNBs. It is not yet clear whether or not these PNBs correspond to coiled bodies (also called sphere organelles in Xenopus oocytes), as decribed by J. Gall (Carnegie Institute, Baltimore). Consistent with this hypothesis was the demonstration that coilin—regarded as a marker protein for coiled bodies—is also present in PNBs. However, P. Bell showed that cellular and in vitro assembled PNBs contain nucleolin, which has not yet been detected in coiled bodies, and that PNBs differ from coiled bodies in their ultrastructure. J. Gall discussed possible functions of the coiled bodies. It may be an initial site of import of splicing components, it may have a role in histone mRNA processing (U7 snRNA is also present), it may be required for or involved in rRNA processing, or it may be involved in some combination of these. Alternatively, coiled bodies could serve

as a storage or recycling organelle for the many components involved in intranuclear RNA metabolism.

Conclusions and Prospects

This meeting has shown that the progress in the field of nucleocytoplasmic transport was substantial in the past few years. This was possible because different experimental approaches dealing with nucleocytoplasmic transport were coming together, including simple and reliable in vitro assays that faithfully measure nuclear protein accumulation and nuclear envelope assembly, microinjection of transport substrates into Xenopus oocytes, biochemical isolation of NPCs or derived subcomplexes, and genetically based assays used to find mutants affecting NPC structure and function. If these multidisciplinary approaches can be further combined and integrated with structural analysis and high resolution light microscopy and EM, we can hope that before the end of this century the basic principles of nucleocytoplasmic transport will be understood and the challenging puzzle of how the 50 different nucleoporins or so that assemble into the 125 MDa NPC could be resolved.

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